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Pathology of Sarcoidosis and Differential Diagnostics of other Granulomatous Diseases

Maria V. Samsonova and Andrey L. Chernyaev

Abstract

Granulomatous diseases are the heterogeneous group of the conditions of different etiologies with a variety of clinic syndromes and morphological features and nonuniform sensitivity to therapy, and the existence of granulomas as general dominate histological expression. Granuloma is indicative of chronic inflammation involving cells of the macrophage system and other inflammatory cells. After the antigen exposure, the activation of T-lymphocytes, macrophages, and epithelioid histiocytes leads to granuloma formation. Granuloma also contains the extracellular matrix produced by fibroblasts, which provide the boundary and isolation of antigen. Their etiology may classify granulomatous diseases as infectious and noninfectious. However, recent studies demonstrate that pathogenic microorganisms may cause the granuloma formation in diseases previously considered as noninfectious. In some cases, differentiation between infectious and noninfectious processes may be problematic. This chapter aims to highlight the multiformity of granulomatous diseases, characterize the pathologic features of different infectious and noninfectious granulomatosis, and delineate the diagnostic approach.

Keywords: lung pathology, granulomatous diseases, sarcoidosis, infection, vasculitis

1. Introduction

Granulomatous diseases are a heterogeneous group of the conditions of various etiologies with a variety of clinic syndromes and morphological features, nonuniform sensitivity to therapy, and the existence of granulomas. Granuloma is indicative of chronic inflammation involving cells of the macrophage system and other inflammatory cells. After antigen exposure, the activation of T-lymphocytes, macrophages, and epithelioid histiocytes lead to granuloma formation. Granulomas also contain the extracellular matrix produced by fibroblasts, which provide the boundary and isolation of antigens. Their etiology may classify granulomatous diseases as infectious and noninfectious. However, recent studies demonstrate that pathogenic microorganisms may cause the granuloma formation in diseases previously considered as noninfectious. In some cases, differentiation between infectious and noninfectious processes may be problematic. This chapter aims to highlight the multiple forms of granulomatous diseases, characterize the pathologic features of different infectious and noninfectious granulomatosis, and delineate the diagnostic approach.

The term granuloma comes from the Latin word “*granulum*” which means “grain,” and the Greek suffix “-*oma*” used to refer to its nodular formation. Granuloma is a nodular defined formation. This term designates compact cell aggregates on microscopy; granulomas may consist of histiocytes and/or epithelioid cells, giant multinucleated cells, and other inflammatory cells (lymphocytes, neutrophils, and eosinophils). Epithelioid and giant multinucleated cells are monocytes/macrophages derivatives; the former represents good differentiated secretory cells, while the latter specializes in phagocytosis [1]. Giant multinucleated cells are formed by fusion or incomplete cell division, proven in experimental studies [2]; moreover, foreign-body giant cells are formed earlier, than Langhans cells. Lymphocytes are located mainly at the periphery of a granuloma and are represented by T-cells while B-lymphocytes are scattered outside the granuloma. Depending on the disease, T-lymphocytes are predominantly represented by T-helpers 1 and 2 or cytotoxic T-suppressors.

Granulomatous diseases are the heterogeneous group of the diseases of different etiology with a variety of clinic syndromes and morphological features, nonuniform sensitivity to therapy [3]. This chapter aims to highlight the variety of granulomatous lung diseases, to characterize the key morphological features of various diseases of infectious and noninfectious nature, as well as to delineate the diagnostic approach. First, the granulomatous diseases, with arising granulomas which do not lead to necrosis development, with some exceptions are mentioned.

2. Sarcoidosis

Lung lesions in sarcoidosis are described in 90% of cases. The morphological feature of sarcoidosis is epithelioid cell granuloma, which is a compact formation of mononuclear phagocytes (macrophages and epithelioid cells). Each sarcoid granuloma has certain stages of development. These stages are as follows:

1. Early or macrophage granuloma, sometimes with a few histiocytes, lymphocytes, or neutrophils (**Figure 1**);
2. Granuloma with an epithelioid cell cluster in the center and macrophages at the periphery;
3. Lymphocytic epithelioid granuloma;
4. The appearance of giant multinucleated cells (at first foreign-body giant cells, followed by Langhans cells);
5. Early cell necrosis in the center of the granuloma due to nuclear pycnosis, the formation of apoptotic bodies, and epithelial cell necrosis;
6. Central fibrinoid, granular, coagulative ischemic necrosis, as a rule, in small foci;
7. Granuloma with fibrosis (or hyalinoses); silver stain is used to detect reticulin fibers;
8. Hyalinized granuloma.

The process of granuloma organization begins at the periphery; that is why they have well-defined, “stamped” appearance (**Figure 2**).

Moreover, in sarcoidosis, granulomas of different “age” may be frequently found in the same samples; granulomas often form conglomerates (**Figure 3**) [4].

A significant number of lymphocytes in lung tissue in patients with sarcoidosis are predominantly represented by T-cells. It is useful to evaluate the bronchioloalveolar lavage (BAL) while carrying out the differential diagnosis: In sarcoidosis

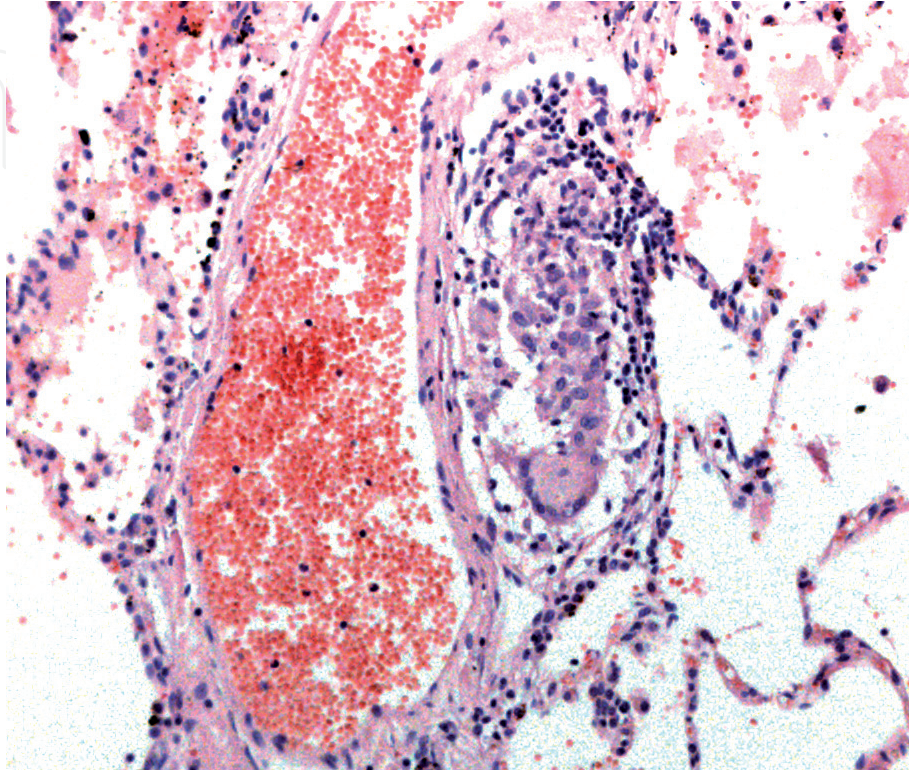


Figure 1.
Macrophage granuloma.

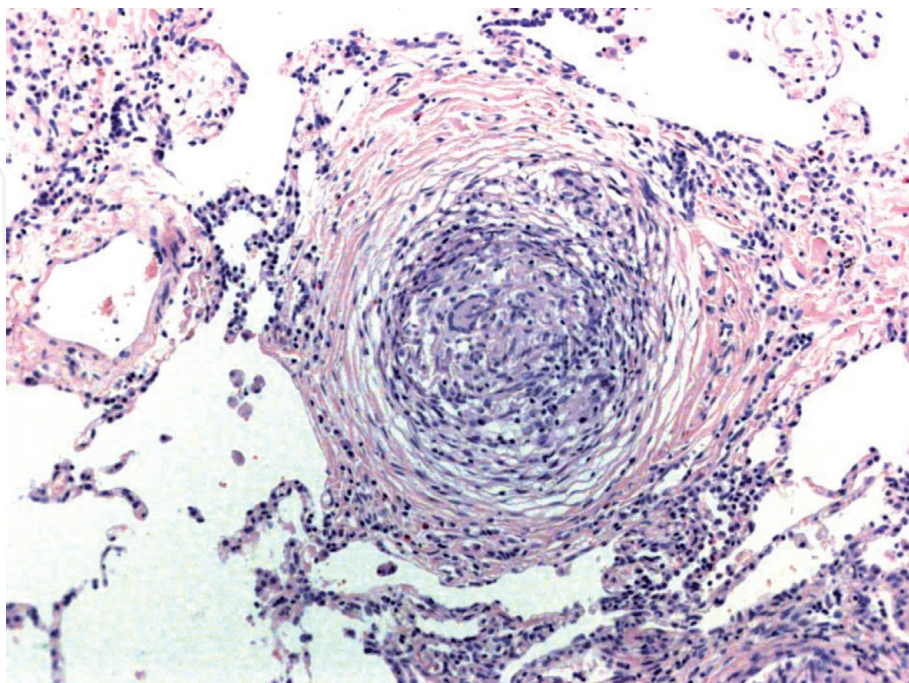


Figure 2.
Typical “stamped” sarcoid granuloma. H&E.

T-helpers predominate. Giant cells in granulomas may contain cytoplasmic inclusions, such as asteroid bodies, Schaumann bodies, or crystalloid structures. These inclusions are characteristic of sarcoidosis, but they are not pathognomonic, as they may be found in other granulomatous diseases [5]. The end stage of sarcoidosis is characterized with prominent fibrosis, sometimes with honeycombing, in which only the remnants of granulomas could be found (**Figure 4**).

Typical locations of granulomas in sarcoidosis are perilymphatic or subpleural zones. Granulomas in bronchi and bronchioles may be found in 15–55% cases of sarcoidosis. Furthermore, granulomas are often located in the vessel wall; the frequency of granulomatous vasculitis may reach 69% (**Figure 5**). In these cases,

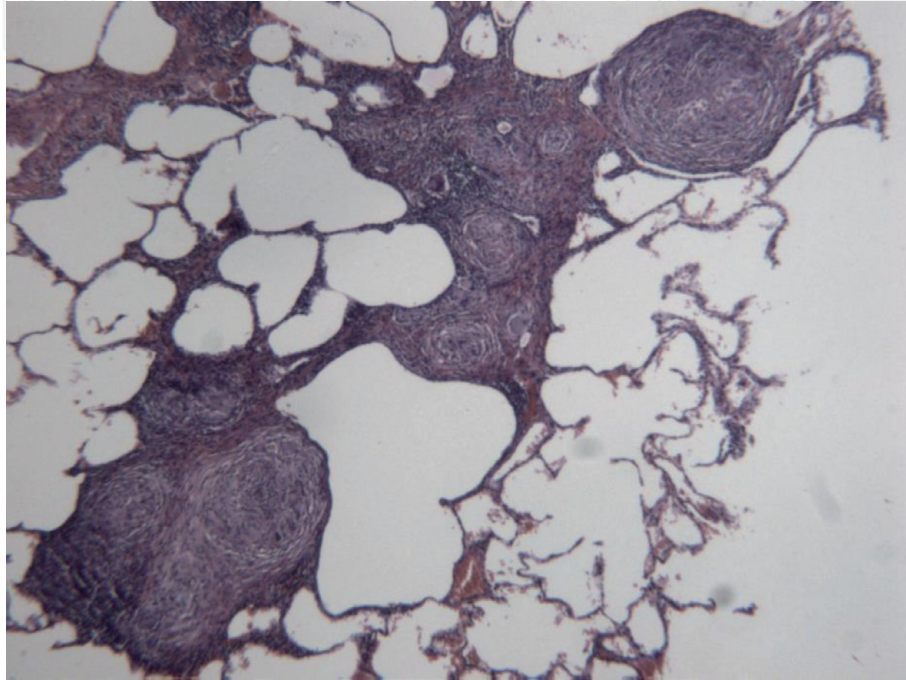


Figure 3.
Multiple well-defined epithelioid cell sarcoid granulomas, forming the conglomerates. H&E.

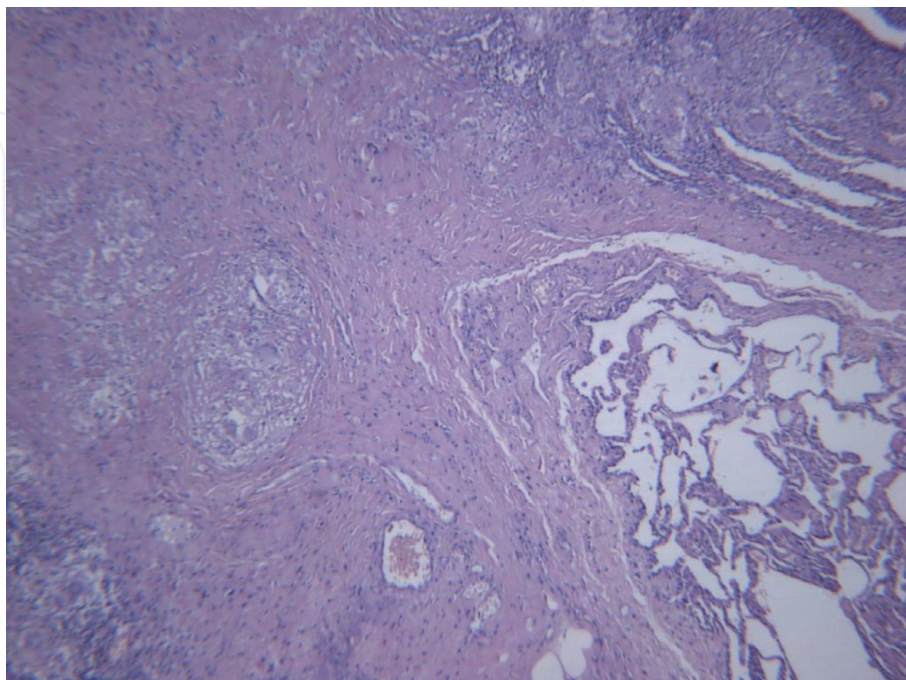


Figure 4.
Late-stage sarcoidosis with prominent fibrosis and remnants of granulomas. H&E.

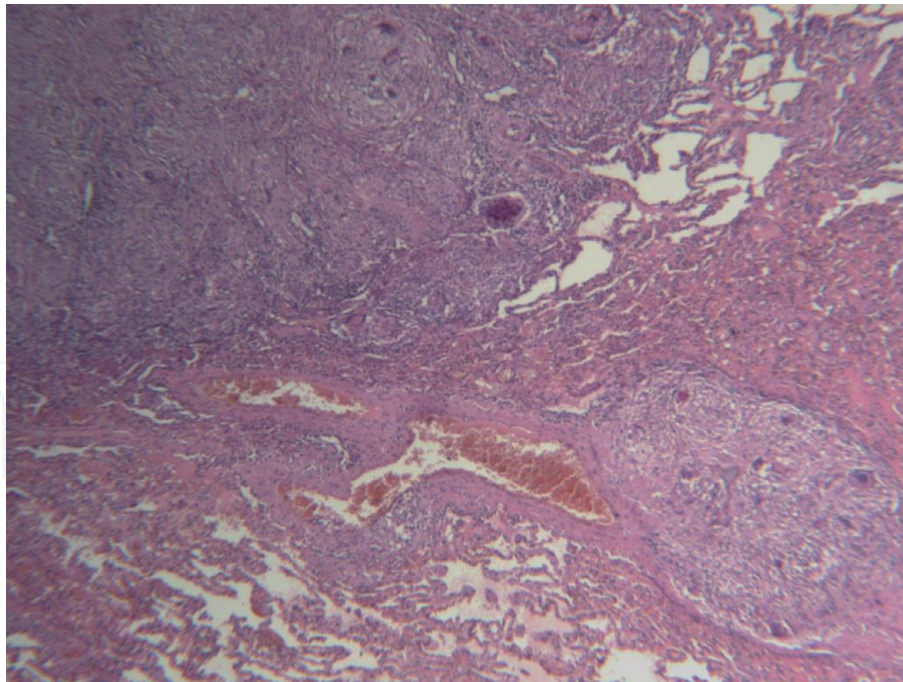


Figure 5.
Granulomatous vasculitis in sarcoidosis. H&E.

sarcoidosis should be differentiated from necrotizing sarcoid granulomatosis. The latter is attributed by some authors to the nodular form of sarcoidosis; necrosis is typical for this disease [6].

Summing up, sarcoidosis is characterized by sharply defined, “stamped” granulomas located along the lymphatic vessels with concentric fibrosis around the granulomas and hyalinosis inside and between the granulomas, also by granulomatous vasculitis, the absence of chronic interstitial inflammation outside the granulomatous lesions, and the absence of organized pneumonia foci. Apart from sarcoidosis, a so-called nonspecific sarcoid reaction occurs in the form of epithelioid cell granulomatosis. It is usually observed in regional lymph nodes, but may also be found in lung tissue in pseudotumors, malignant neoplasms, parasitic diseases, and tuberculosis. Histologically, the sarcoid reaction is characterized by its locality and a topical relationship with these pathological processes.

2.1 Hypersensitivity pneumonitis

The pathogenesis of hypersensitivity pneumonitis is based on type III (immune-complex) and type IV immunological reactions in the lung during allergen inhalation. The etiological factor of this medical condition is usually thermophilic bacteria, fungi, and animal proteins. Other bacteria and their products, amoeba, and some chemicals are much less likely to cause the disease. In case of hypersensitivity pneumonitis, granulomas are poorly formed, and loose, composed of histiocytes, lymphocytes and, multinucleated cells; some eosinophils may also be identified (**Figure 6**) [7].

Unlike sarcoidosis, peribronchiolar localization of granulomas is typical for hypersensitivity pneumonitis. The triad of pathomorphological features characterizes hypersensitivity pneumonitis: Nonspecific interstitial pneumonia in the peribronchiolar zones, non-necrotizing histiocytic (giant cell) granulomas, and foci of bronchiolitis obliterans. “Needle-like” inclusions are often observed inside giant cells in the granuloma areas and the alveoli in hypersensitivity pneumonitis. In the late, fibrous stage of the disease, the histological features are similar to those in

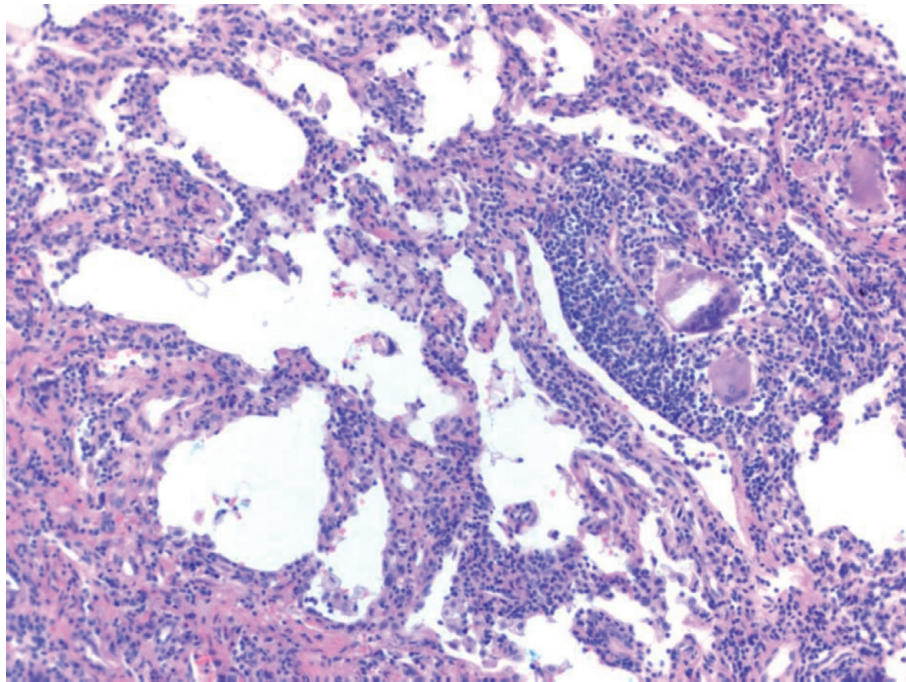


Figure 6.
Hypersensitivity pneumonitis: Ill-defined granuloma consisting of giant multinucleated cells with needle-like structures surrounded by lymphocytes, lymphocytic infiltration of interalveolar septa. H&E.

usual interstitial pneumonia, only scattered giant cells or remnants of granulomas may be found in the fibrotic areas or in the honeycomb zones in hypersensitivity pneumonitis [8].

2.2 Chronic allergic disease caused by metals

Chronic berylliosis is an allergic granulomatosis. Granulomas are similar to those in sarcoidosis and may be slightly larger. As in sarcoidosis, granulomas have perilymphatic location, and lymph nodes are usually affected. The same variant of granulomatosis may develop as a result of zirconium exposure. The diagnosis should be based on the clinical history and lymphocyte transformation test [9].

2.3 Polyangiitis with granulomatosis

In our opinion, of particular difficulty is **the differential diagnosis of necrotizing granulomatosis**. Infectious disease, as one of the most common causes of necrotizing granulomatosis, should be differentiated from polyangiitis with granulomatosis (previously Wegener's granulomatosis), aspiration pneumonia, and less often from nodular form of rheumatoid arthritis, necrotizing sarcoid granulomatosis (NSG), lung infarction, and lymphomatoid granulomatosis [10]. Despite the specific histological changes in these diseases, there is, however, an overlap, and only a combination of histological features help in the final diagnosis. **Infectious necrotizing granuloma** usually has smooth contours, commonly eosinophilic necrosis, surrounded by a rim of histiocytes and giant multinucleated cells. By contrast, in polyangiitis with granulomatosis, the necrosis zone has uneven contours resembling a geographical map, with a large amount of cellular debris, which gives the necrosis a "dirty" appearance (**Figure 7**).

Necrotic areas are also surrounded by a histiocytic rim; however, giant cells are usually few in number and are scattered without forming compact granulomas. A characteristic feature of polyangiitis with granulomatosis is the necrotizing vasculitis with fibrinoid necrosis of the media (**Figure 8**);

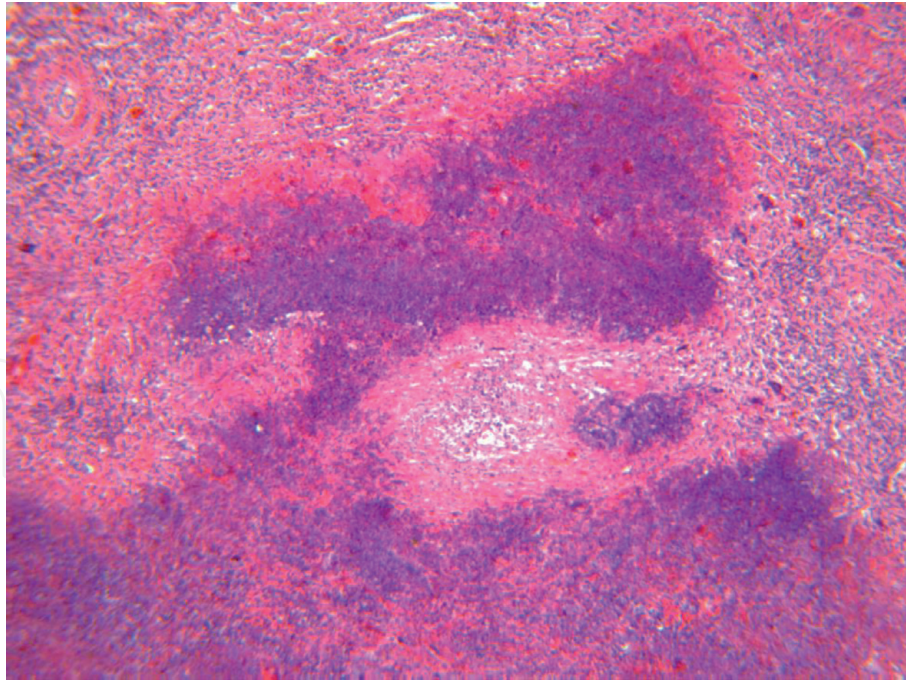


Figure 7.
Polyangiitis with granulomatosis: "geographic" necrosis with cell debris. H&E.

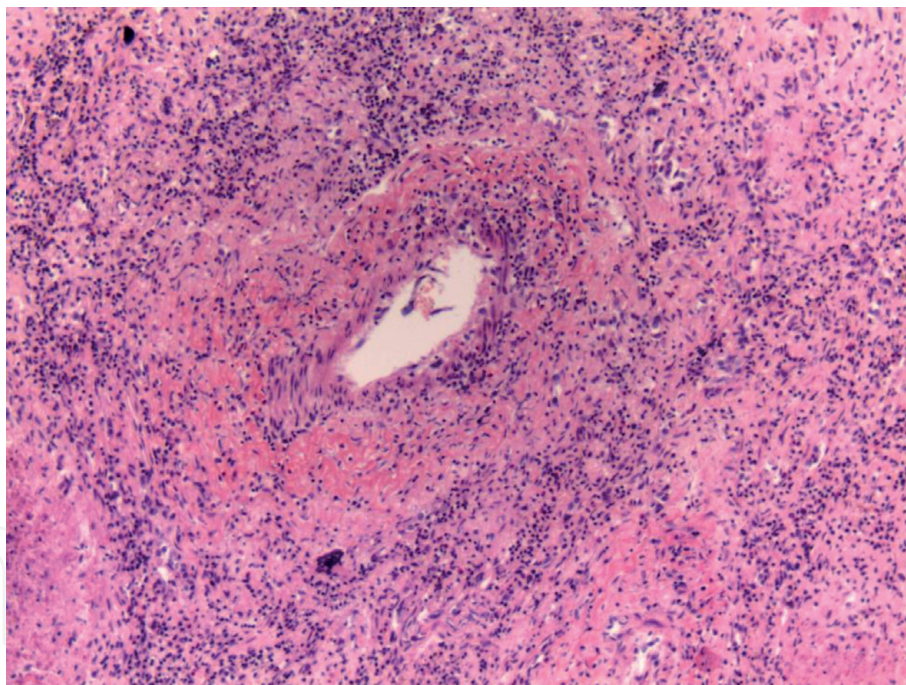


Figure 8.
Polyangiitis with granulomatosis: Fibrinoid necrosis of the vessel wall with lymphocytic infiltration. H&E.

Such vessels are observed in the inflammation; the damage to the vessel wall is often eccentric; also, the branches of the pulmonary arteries and veins are affected in this disease. The capillaritis, accompanied by intra-alveolar hemorrhages, may also be observed. Necrotizing vasculitis may often be found in the inflammation and necrosis, but this is also in the infectious granulomatosis; therefore, to confirm the diagnosis of polyangiitis with granulomatosis, it is necessary to carefully evaluate the vessels outside necrosis, and additional stains are recommended to identify elastic tissue (Verhoeff-van Gieson stain and others). In contrast to infectious granulomatosis, lymph node involvement is not typical for polyangiitis with granulomatosis [11].

2.4 Allergic angiitis with granulomatosis

Necrotizing granulomatosis is also seen in (previously, Churg-Strauss syndrome), in combination with necrotizing vasculitis and eosinophilic pneumonia. Granulomas in allergic angiitis with granulomatosis are well formed; with central necrosis containing many eosinophils, there is also eosinophilic infiltration of the blood vessel walls and bronchioles, necrotizing vasculitis with eosinophils, and giant multinucleated cells present. However, additional clinical and laboratory data are necessary to determine the diagnosis, because the classical triad (granulomatosis, necrotizing vasculitis, and eosinophilic pneumonia) is rarely found in lungs.

2.5 Rheumatoid arthritis

Furthermore, necrotizing pulmonary granulomas may form in rheumatoid arthritis; however, the diagnosis in this case should be made with caution. First of all, one should take into account clinical data, since nodular forms of rheumatoid arthritis develop only in the active phase in seropositive patients with severe articular syndrome. As a rule, necrosis is eosinophilic, cell debris is usually located between the necrosis and the surrounding rim of histiocytes; it may be combined with vasculitis, but necrotizing vasculitis is not characteristic for this disease (**Figure 9**) [12]. The described histological features are practically indistinguishable from infectious granulomatosis; moreover, rare clinical cases of rheumatoid arthritis and tuberculosis combination have been described, and therefore, the infection must be thoroughly excluded.

2.6 Necrotizing sarcoid granulomatosis (NSG)

Infectious granulomatosis should also be differentiated from NSG. Some characteristics of the latter are likened to those seen in polyangiitis with granulomatosis. NSG is characterized by the interstitial necrosis, which is often eosinophilic, but

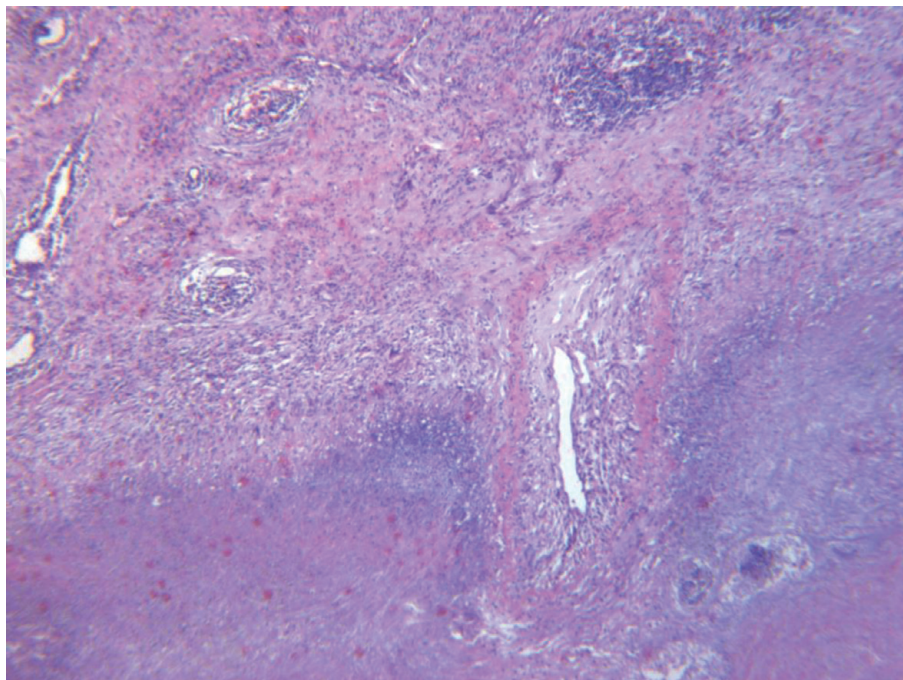


Figure 9. *Rheumatoid arthritis: Extensive necrosis with rims of histiocytes at the periphery and lymphoid infiltration of vessel wall. H&E.*

sometimes may contain cellular debris. However, necrosis in NSG is combined with the non-necrotizing sarcoid-type granulomas, consisting mainly of epithelioid and giant multinucleated cells with just a small number of lymphocytes. These granulomas tend to merge and are often located near blood vessels or in its walls, but not causing vasculitis (**Figure 10**) [6]. The following symptom triad is obligatory for the NSG diagnosis: sarcoid-type granulomas, granulomatous vasculitis, and necrotizing inflammation.

Since necrosis in infectious granulomatosis may be of a coagulation type, pulmonary infarction should also be a part of the differential diagnosis. In the stage of organization, infarction may be surrounded by fibroblasts and inflammatory cells, resembling granulomatous inflammation. As a rule, in lung resection specimens with pulmonary infarction, thrombi can be detected in the branches of the pulmonary artery that caused the development of a pulmonary infarction [13].

2.7 Granulomatous inflammation caused by infectious agents

2.7.1 Fungi

Fungi that cause deep mycoses, as a rule, do not form granulomas in the lungs. In most cases, fungi such as *Aspergillus*, *Candida*, and some others cause local mycetoma, diffuse invasive mycosis, or allergic reactions (allergic bronchopulmonary aspergillosis/mycosis). The granulomatous response in these fungi infections is rare [14].

2.7.1.1 Histoplasmosis

Histoplasmosis is caused by *H. capsulatum* (North America, river valleys) and *H. duboisii* (Africa), which are budding yeast cells with a diameter of 2–4 μm . Microorganisms are found in the cytoplasm of macrophages, histiocytes, and necrotic debris. Its capsule is stained with Giemsa or PAS reaction. Both organisms

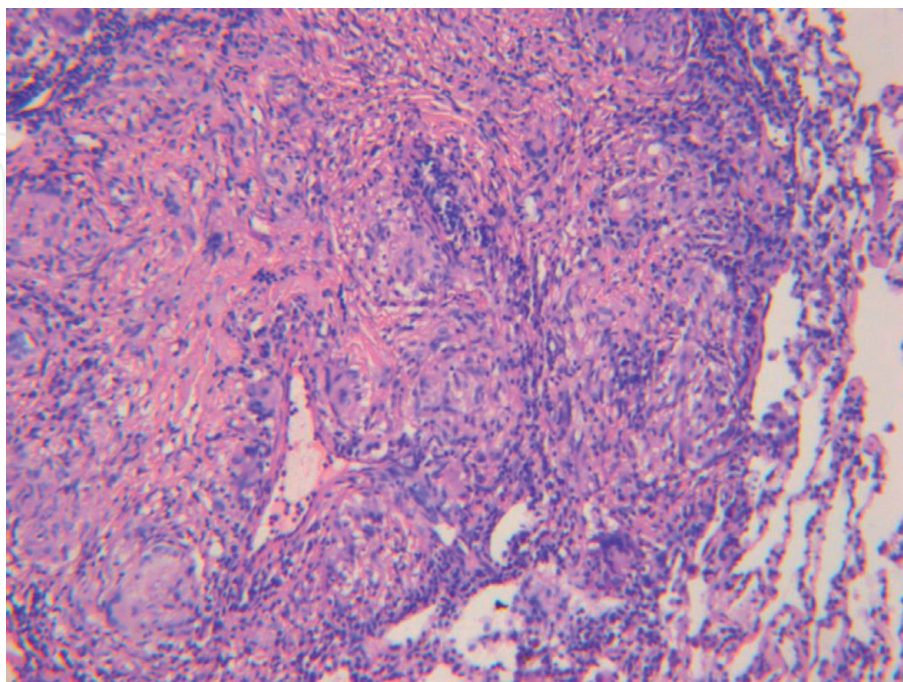


Figure 10.
Necrotizing sarcoid granulomatosis: Granulomatous vasculitis. H&E.

cause the formation of epithelioid cell granulomas; however, necrotizing granulomatosis is more often described in *H. capsulatum* infection [15].

2.7.1.2 *Cryptococcosis (European blastomycosis)*

Cryptococcus neoformans is ubiquitous; it is found in soil and pigeon excrements. The fungal cell is of 4–7 μm in size, replicates by budding, and is stained with H&E, mucicarmin, and PAS. Cryptococci cause a spectrum of various changes in the lungs. A typical granulomatous reaction presents with confluent non-necrotizing granulomas, many multinucleated giant cells, and a mild inflammatory reaction; giant cells are located mainly outside the granulomas and contain cryptococcal cells (**Figure 11**).

These fungi can also be located inside necrotizing granulomas (cryptococcomas) resembling those with mycobacterial and other types of fungal infections (**Figure 12**). In immunocompromised individuals, cryptococcal cells are found inside the alveoli, in their walls, and in the interstitium, without marked inflammatory reaction; some scattered multinucleated giant cells can be found [9].

2.7.1.3 *Coccidiosis*

Coccidia most often lead to necrotizing granuloma formation; the eosinophilic reaction may be marked or absent; numerous neutrophils may be observed. Like in other infections, granulomas are located peribronchiolar or with destroyed bronchioles. This process is accompanied by the formation of small non-necrotizing granulomas at the periphery. *Coccidia* are usually found in the center of necrotizing granulomas, they consist of large spherical structures (spherules) containing yeast-like structures (endospores). Endospores of various sizes can be located in necrosis or cellular debris, resembling other fungal infections. The detection of spherules and endospores favor the diagnosis of coccidiosis. Like *Histoplasma*, *Coccidia* do not grow in vitro; thus, the diagnosis can only be made by histological examination [15].

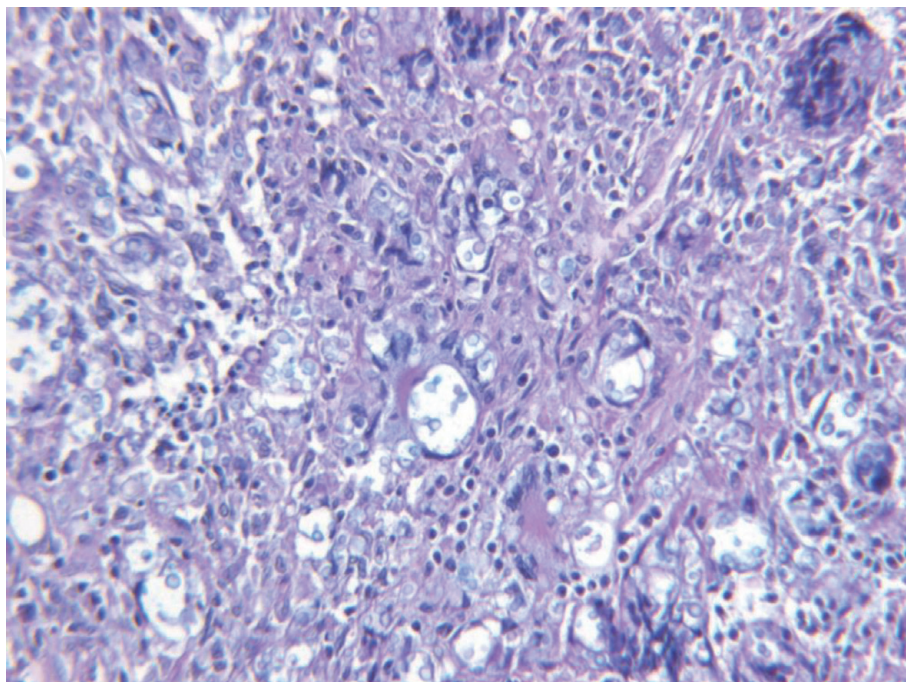


Figure 11. *Cryptococci cells and multinucleated giant cells in a granuloma. Combined stain of alcian blue and PAS.*

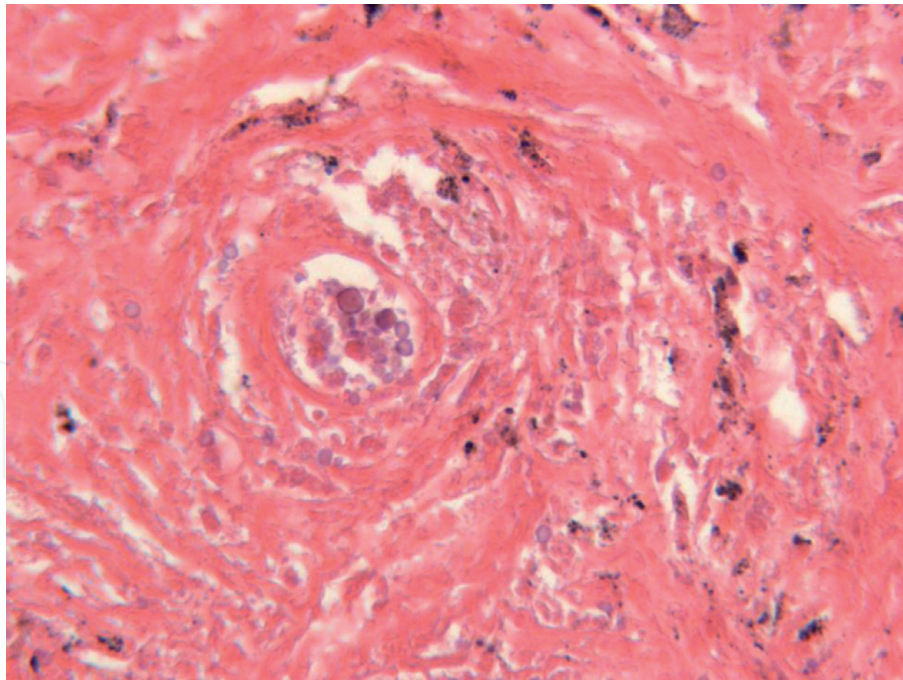


Figure 12.
Necrotizing granuloma fragment with cryptococci cells. H&E.

2.7.1.4 Blastomycosis

Blastomycosis is a rare disease, and this diagnosis may be suspected when granulomatosis or giant cell lesion in combination with severe acute inflammation is detected. Blastomycosis is characterized by basophilic necrosis rich in cellular debris in contrast to eosinophilic or slightly “dirty” infectious necrotizing granulomas. Blastomycosis is often a bronchiolocentric process. Large, thick-walled, yeast-like *Blastomyces* cells can be detected in H&E and also mucicarmine staining. Active budding is a distinctive feature of the microorganism; nuclear material (multiple nucleoli) can also be found inside the cells, but these signs are not always observed. In this regard, compared to *Histoplasma* and *Cryptococci* cells and *coccidial* endospores, *Blastomyces* cells are larger but still smaller than *coccidial* spherules [15].

2.7.1.5 Pneumocystosis

Most pathologists are familiar with the conventional picture of pneumonia caused by *Pneumocystis*, but 5–17% describe the formation of an ill-defined intraalveolar epithelioid or histiocytic granuloma around the eosinophilic exudate, sometimes without exudate; the formation of well-defined granulomas with central necrosis or without it is also possible [16, 17]. Sometimes the granulomatous reaction in pneumocystis pneumonia is a foreign-body granulomatosis (**Figure 13**).

2.8 Parasites

Dirofilaria is one of the most common parasites that lead to the granulomatous inflammation in lungs. This nematode infects dogs more commonly, but the disease can also occur in humans, as the infection is transmitted through an insect bite; the larva enters the right heart and into the pulmonary arteries during embolism, causing thrombosis of the latter with an infarct-like necrosis development (**Figure 14**). In one-third of the cases, granulomas are formed in the adjacent lung

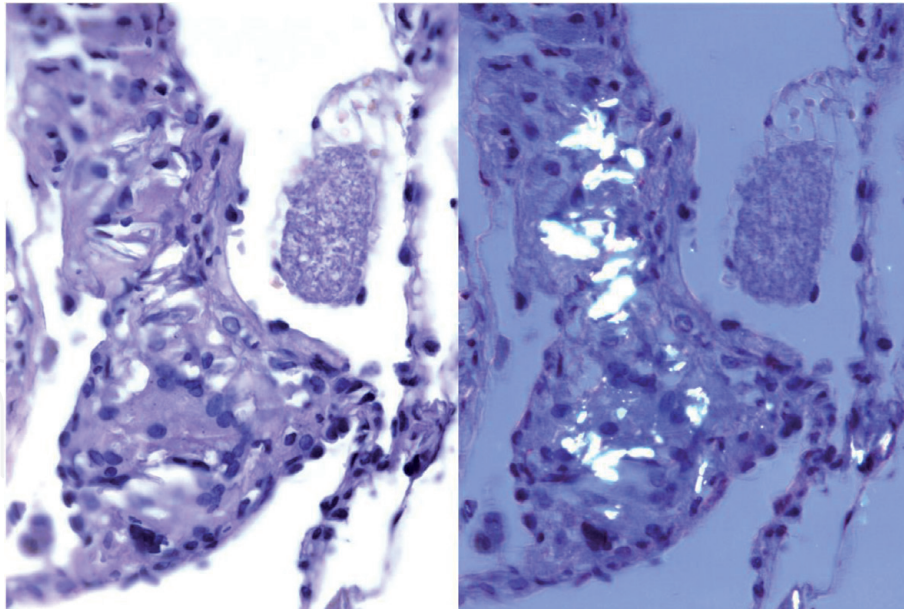


Figure 13. Perivascular foreign-body granuloma (talcosis) in a case of pneumocystic pneumonia in a drug abuser. H&E in bright field versus polarization.

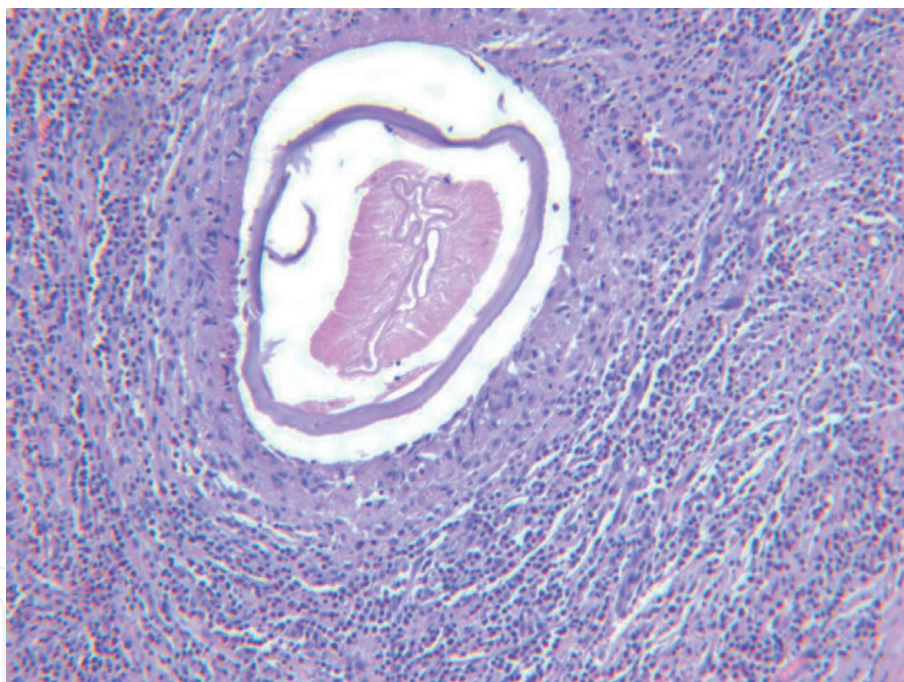


Figure 14. *Dirofilaria immitis* larva in the pulmonary artery, infiltration of plasma cells and eosinophils. H&E.

tissue, necrotizing or non-necrotizing vasculitis is observed in half of the cases, and two-thirds of the cases demonstrate eosinophilic infiltration [18].

2.9 Tuberculosis

Tuberculosis is caused by members of the *Mycobacterium tuberculosis* family, namely, *M. tuberculosis*, *M. bovis*, and *M. africanum*, which belong to the group of rapidly growing mycobacteria. The virulence of these microorganisms varies from moderate to highly virulent strains. Changes observed in the lungs in tuberculosis patients can be very diverse ranging from common necrotizing granulomas, miliary necrotizing granulomas to non-necrotizing granulomas, tuberculoma, and healed

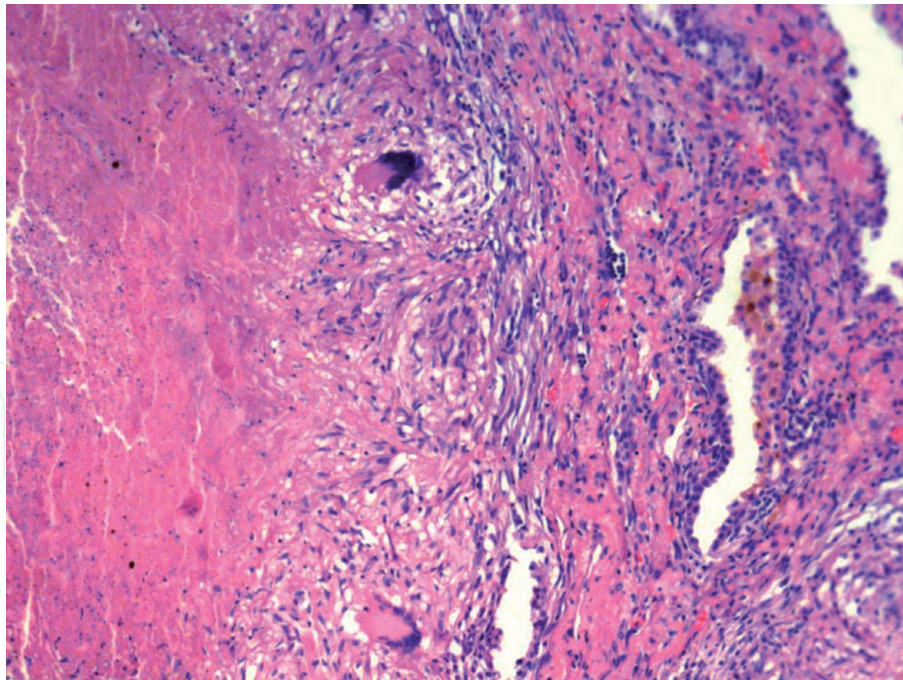


Figure 15.
Tuberculous necrotizing granuloma with lymphocytes, histiocytes, and giant multinucleated Langhans at the periphery. H&E.

fibrous granulomas, all of these depend on the virulence on the one hand and the immune defense status on the other (**Figure 15**) [19]. Granulomas in tuberculosis usually have a bronchiolocentric localization, but it should be kept in mind that this could be the case in any infectious granulomatosis and even in sarcoidosis. Histological features in tuberculosis are indistinguishable from those in non-tuberculous granulomatosis. This was confirmed in a study by Corpe and Stergus, in which 27 pathologists, specializing in the mycobacterial disease diagnostics, were asked to evaluate 25 histological slides without information about culture-confirmed infection. In most cases, it was not possible to distinguish between tuberculosis and mycobacteriosis [20]. Thus, the tuberculosis diagnosis **should be based** on the identification and subsequent determination of the type of *Mycobacterium*.

2.10 Non-tuberculosis mycobacteriosis

Non-tuberculosis mycobacteriosis is an inflammation caused by mycobacteria not belonging to the *Mycobacterium tuberculosis* family; those are *M. avium*, *M. fortuitum*, *M. gordonae*, *M. kansasii*, *M. xenopi*, and *M. marinum*, also designated as MAC complex. Unlike *Mycobacterium tuberculosis*, these mycobacteria can be detected intracellularly in macrophages (histiocytes), and they can be numerous in immunocompromised individuals. The diagnosis is made based on acid-fast stain, cultural or molecular biological tests. As mentioned above, the histological changes are often similar to those in tuberculosis. Non-necrotizing granulomas, histiocytic granulomas, and granulomas consisting of foamy and granular macrophages containing mycobacteria can also be detected. Solovieva et al. have described the following spectrum of histological changes in mycobacteriosis:

- tuberculous granuloma—epithelioid cell tissue, variable number of Langhans cells and the necrosis intensity, few mycobacteria;
- a reactive, necrotizing multibacillarity—weak inflammatory response, abundance of mycobacteria in the necrosis area;

- multibacillary histiocytosis—diffuse macrophage infiltration with an intracellular abundance of mycobacteria, no necrosis;
- multibacillary minimal histiocytosis—a mild inflammatory reaction with an intracellular abundance of mycobacteria;
- histoid lesion—nodular clusters of spindle-shaped macrophages with an abundance of mycobacteria;
- nonspecific granulation tissue;
- acute purulent abscess [21].

The MAC-hypersensitivity-like disease has also been described (or “hot tub lung”); it is caused by mycobacteria of the MAC complex, and associated with the use of sauna and showers, which leads to the aerosol inhalation. The histological features of this disease are similar to the hypersensitivity pneumonitis changes [22].

2.11 Differential diagnostics of granulomatous lung diseases

A variety of diseases leading to granulomatosis determine certain difficulties in conducting the differential diagnostics even when resectional (surgical, video-assisted) biopsies are performed, which allows obtaining a sufficient amount of material for histological evaluation [23]. However, it is not always possible to establish the cause of granulomatous inflammation. According to Ulbright and Katzenstein, who analyzed 86 solitary lung granulomas detected by X-ray, the infection caused by acid-resistant mycobacteria or fungi was confirmed in 70%. In 25 cases, the infectious etiology was not proven, while two patients were diagnosed with hyalinized granuloma, one patient—with polyangiitis with granulomatosis, and in 22 cases, it was not possible to classify the process. Also, a significant similarity of histological changes in infectious granulomas and polyangiitis with granulomatosis was found; it may be possible that the latter was a reflection of the immune response disorders to an infectious agent which could no longer be found in the tissue samples. This means that polyangiitis with granulomatosis and other lung angiitis diagnoses should be made with extreme caution in cases with solitary nodes while no damage to other organs is detected. In such cases, a thorough examination of patients and follow-up should be recommended [24].

Mukhopadhyay et al. conducted a multicenter retrospective study of 500 biopsies from 10 clinics in the United States, Britain, Austria, Brazil, Japan, Turkey, and India with pulmonary granulomatosis. During the biopsy analysis, a specific diagnosis was established in 58% of the cases: most commonly sarcoidosis (27%) and mycobacterial infection (25%) were detected. Mycobacterial infection was proved in 18% outside the USA versus 8% in the USA; on the contrary, fungal infection amounted to 19% in the USA (most often histoplasmosis) versus 4% in other countries. Fungi were commonly detected by histological examination, while mycobacterial infection was confirmed in culture. In 42% of the cases, the etiological factor of granulomatosis was not established.

This study, in our opinion, is extremely interesting: First of all, it indicates the predominance of sarcoidosis and infectious granulomatous inflammation in the structure of granulomatous diseases according to histological analysis conducted in different countries and geographical regions. Fungal infection more often caused granulomatous inflammation in the United States, while mycobacterial infection was more often diagnosed in other countries, which is a reflection of the infection

endemicity. It is crucial to send the specimen simultaneously to the histological and microbiological laboratory in all cases when a granulomatous disease is suspected, that will definitely improve the quality of the etiological diagnosis. According to this study, the cause of granulomatosis was not established in more than a third of clinical cases even after histological examination [22].

The frequency of infectious granulomatosis is high. When all other causes based on the clinical history, clinical syndromes, laboratory tests, and specific morphological features mentioned above are excluded, the remaining granulomatous diseases are most likely to be attributed to infectious.

An important issue in the differential diagnosis of the infectious granulomatosis diseases is the detection of an infectious agent in microscopical slides. To achieve this, it is necessary and obligatory to perform additional stains. One can detect fungal infection, first of all, by carefully evaluating hematoxylin- and eosin-stained (H&E) slides. Most fungi, such as *Cryptococcus*, *Blastomyces*, *Coccidioides*, and *Aspergillus*, can be found in H&E slides, more often in necrosis areas than in the adjacent lung tissue. The pathologist should select slides with necrosis present when ordering additional stains. Grocott's methenamine silver stain and the PAS reaction are the most commonly used for the fungal infection diagnosis confirmation, also alcian blue (Mouri), the basic brown stains (Shubich's method), or the combined stain of PAS with alcian blue [14].

Ziehl-Neelsen stain is used to diagnose mycobacterial infection; however, mycobacteria are usually few in number, and their search is quite time-consuming. Alternative stains with auramine or auramine/rhodamine increase the sensitivity of the method, but these techniques require fluorescence microscopy (**Figure 16a** and **b**). To increase the detection of mycobacteria, Ulbright and Katzenstein recommend performing staining in at least two blocks [24]. Gomori silver or Warthin-Starry stains are recommended for syphilis diagnosis [9].

One of the open questions is whether there are additional opportunities, modern techniques for diagnosing infectious granulomatosis. Immunohistochemistry is available, but it has several limitations, namely cross-reactivity, as well as the antibody accessibility, especially for rare microorganisms. *In situ* hybridization may

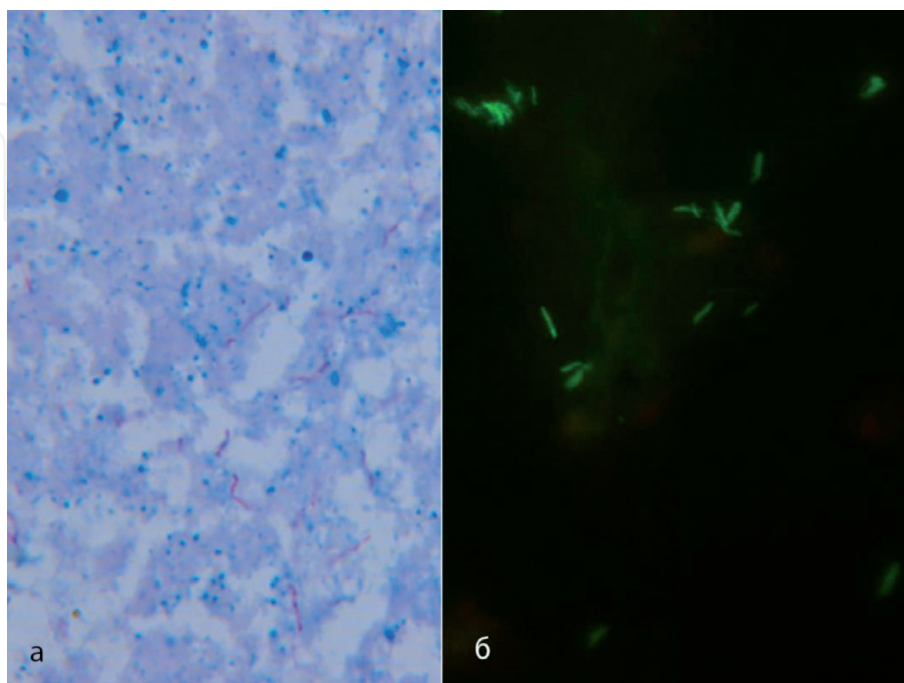


Figure 16.
Acid-fast mycobacteria: (a) Ziehl-Neelsen stain and (b) auramine/rhodamine (fluorescence).

also be useful, but not for the detection, but for the identification of fungi found in traditionally stained slides. Real-time PCR can be performed on paraffin sections for tuberculosis diagnostics; the specificity of this method is 99%; however, the sensitivity amounts to only 65%. Nevertheless, when the same method is used to detect mycobacteria tuberculosis in the cerebrospinal fluid, urine, or bronchoalveolar lavage, the sensitivity is more than 90%. Thus, this technique is useful and confirms the diagnosis if mycobacteria are detected, but PCR does not exclude the tuberculosis diagnosis if the result is negative. In addition, it does not allow detecting non-tuberculous mycobacteria. According to Aubry, the cultural study remains the “gold standard” for non-tuberculous mycobacteriosis diagnostics, and, according to their research, indicates that in more than 75% of cases, the only method to confirm mycobacteriosis was in culture [10].

The etiological factor remains unclear in 30–40% of infectious granulomatosis even in leading US university clinics despite a complete histological evaluation of lung tissue slides, as well as correlations with clinical, microbiological, and serological data. In this regard, the question of diagnostic significance of these diseases arises. Ulbright and Katzenstein propose that such cases represent infectious granulomas in which the microorganism was destroyed and/or removed by means of the developed inflammatory process [24]. A retrospective analysis of necrotizing granulomas showed that patients who did not receive further specific therapy were still alive and did not demonstrate any clinical symptoms. The same hypothesis is confirmed by Aubry who notes that even if new foci appear in these patients, poor outcomes were not detected [10]. It can be recommended to pathologists to give a descriptive histological conclusion indicating the presence/absence of necrosis, the absence of detected microorganisms: “The disease etiology is most likely to be infectious, special stains for the microorganism detection are negative.”

When a granulomatous disease is suspected and lung resection is performed, it is necessary to save some specimen tissue unfixed for possible cultural study, and use the quick freeze method at -70°C for subsequent DNA and RNA analyses, if available. In the differential diagnosis of granulomatous diseases, first of all, one should determine whether the granuloma is infectious, or there are signs of other diseases, including Wegener’s granulomatosis. If a specific diagnosis is excluded, it is crucial to perform special stains for microorganism detection, it is preferable to stain sections from at least two blocks, while making sure that necrosis foci are present in the material. If the microorganism detection is not possible at the first glance, we recommend evaluating the slides again at a higher magnification, and also using an additional block for staining. When negative result is received, but clinical data are in favor of tuberculosis or other infections, PCR is suggested. Additional cultural and serological studies, which would be able to exclude the infectious process, should be performed in case of another negative result obtained. Nevertheless, according to this algorithm results, a certain part of granulomatous diseases appears to have an uncertain etiology.

In conclusion, we would like to emphasize that the differential diagnosis of granulomatous pulmonary diseases is not so easy for pathologists. To exclude or prove the infectious disease, the pathologist should carefully examine special stained specimens. Noninfectious granulomatous lung disease should be proved, taking into consideration both clinical and radiological data. Finally, from the pathologic point of view, there are the situations for which a specific diagnosis cannot be made. Multidisciplinary approach sometimes is recommended for decision-making.

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